Short Communication

Crazy Sepal: A New Floral *Sepallata*-like Mutant in the Wild Potato *Solanum microdontum* Bitter

John Bamberg

USDA/Agricultural Research Service, US Potato Genebank, 4312 Hwy 42, Sturgeon Bay, WI, 54235, USA
Tel: 920-743-5406; Fax: 920-743-1080; Email: nr6jb@ars-grin.gov
Web site: http://www.ars-grin.gov/ars/MidWest/NR6/

ABSTRACT

The major economic impact of features of reproduction in *Solanum* and its close relatives make basic study of the genetics and physiology of flowering important in this genus. A qualitative floral-development mutant in the wild potato *Solanum microdontum* Bitter (PI 473166) was discovered that is ostensibly normal in all respects except that repeated (indeterminate) whorls of strap-like sepals replace petals, pistil, and anthers, thus called "crazy sepal" = *cs1*. This mutant appears to be similar to *sepallata* mutants in Arabidopsis. Mutant clones vary in the development of sepals (ranging from small and light colored to larger, dark, and leaf-like) and the determinance of whorls. Crossing studies support single-locus recessive inheritance. This mutant may be a useful tool for the study of floral genetics in *Solanum*, potential pleiotropic physiological effects on tuberization, and other features of the potato crop. It would be a good marker for the study of rare genetic events since individuals reverting to flowering would be very easily detected among thousands of their mutant sibs. Transgene escape is of concern in potato, especially in Latin America where compatible wild species often grow in proximity to the crop. The absolute sterility of this mutant could be applied to this and similar practical breeding problems.

INTRODUCTION

The major Solanaceous crops tomato, pepper, eggplant and ornamentals like petunia are dependent on flowering and sexual reproduction. While potato is mostly a clonally propagated crop, breeding depends on sexual reproduction, as does the study and introgression of wild, seed population-based exotic germplasm of related species.

"Homeotic" mutants that alter the identity of organs (Ditta et al. 2004) have been of great interest for many years, since they provide unique insights into the basic steps underlying normal morphological development (Eckhart 2003). Genes that confer floral identity to meristems and specific flower organ identity in meristematic whorls have been described. These include *sepallata* genes identified in Arabidopsis that determine sepal identity in the first floral whorl, and, in certain mutant genotypes, can result in flowers being replaced by repeating whorls of sepals. Similar genes have been identified in tomato and petunia. Krizek and Fletcher (2005) review these and other recent advances in the genetics of flower development. Hart and Hannapel (2002) implicated a MADS box POTM1 gene in floral development of meristems in potato.

Three mutant plants with no petals were noticed among their 18 normal sibs during routine seed regeneration of the *Solanum microdontum* population PI 473166 at USPG. This population is native to Salta Province, northern Argentina.

Over 100 populations are kept at USPG (see USPG Web site for details). When 12 plants each of all available *S. microdontum* populations were planted in the greenhouse for an unrelated experiment, crazy sepal (cs) was only observed in population 473166, suggesting that the mutant is rare. The species *microdontum*, although non-cultivated and diploid is a close relative of cultivated *tuberosum*, and has been incorporated into the breeding pool, primarily for its late blight (*Phytophthora infestans*) resistance (e.g., Bisognin et al. 2005). Complete information on the source material for this mutant and other germplasm is available through the Germplasm Resources Information Network Web site (http://www.ars-grin.gov/npgs/index.html).

The name "crazy sepal" with allele = *cs1* was applied, referring to the apparent misplaced and overproduced sepals (Figure 1).

MATERIALS AND METHODS

Crazy sepal mutant inflorescences were examined under a dissecting microscope.

Crosses were made to characterize the inheritance of the mutant in *microdontum* PI 473166, with an initial hypothesis of single-locus recessive. Obviously, no typical analysis using testcross matings involving cs mutant phenotypes were possible, since the mutant form is completely male and female sterile. The population from which cs was originally observed was replanted in the greenhouse. Assuming *cs1* to be recessive, only heterozygous female parents carrying the *cs1* mutant allele would produce cs progeny. Female parents identified as *cs1* carriers were pairwise mated. Resulting progenies were separately planted in the field and scored for segregation of cs phenotypes. Observations of variation of expression of cs mutants in the field were also made.

RESULTS AND DISCUSSION

Microscopic observation of cs mutant inflorescences showed them to be derived from simple repeating (indeterminate) whorls of only sepals.

When a new sample of seedlings of PI 473166 was reared in the greenhouse, 21 of 131 plants had the cs phenotype, or about 16%. If at equilibrium, that implies a single recessive *cs1* allele frequency of 40%, and therefore a 48% frequency of heterozygous plants. Eliminating double recessive cs mutants
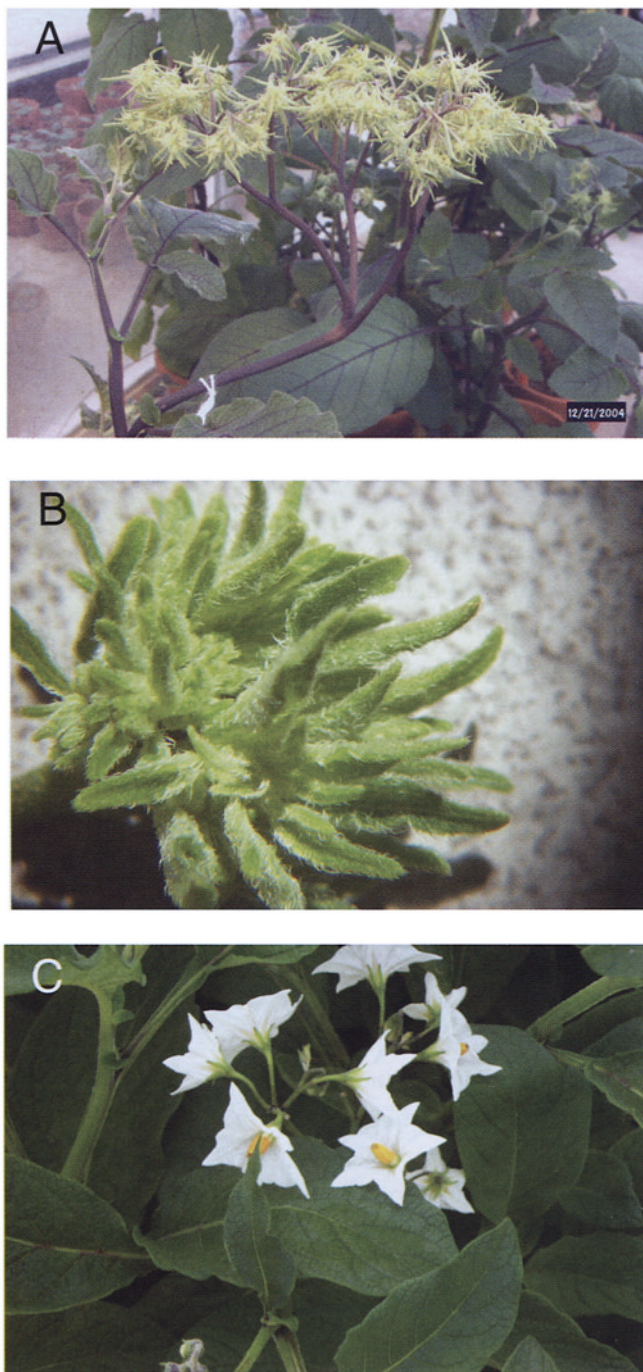
FIGURE 1.
Crazy Sepal inflorescence (A), close-up of *Crazy Sepal* bud (B), and normal inflorescence (C).

results in an expected 57% frequency of heterozygote and 43% homozygotes among normal phenotypes. However, when the 110 normal plants were grown for intermating, the 36 female parents that produced sufficient seed to be genotyped revealed many more than expected heterozygotes: 27 (75%)

heterozygotes (carriers of the mutant allele) and 9 (25%) homozygous non-carriers. Perhaps heterozygotes have more fecundity. If so, that form of reproductive fitness advantage might explain why the cs1 allele, being a reproductive lethal, has not been purged from the population.

Female parents of original PI 473166 seedlings identified as heterozygotes would contribute 50% cs1 eggs. Assuming the detected frequency of heterozygotes among normal phenotypes (75%) for pollen parents, we expect 37.5% frequency of cs1 pollen. Thus, 50% × 37.5% = 18.75% expected cs offspring from known heterozygous female parents following a bulk intermate of the normal plants. With ~19% cs progeny and 81% normal progeny, detection of heterozygote female parents was assured to >99% probability by observation of 30 offspring (i.e., observation of any cs progeny indicated a heterozygous female parent, and no cs progeny in a sample of at least 30 progeny indicated homozygous wild type female parent). The average frequency of cs progeny in the 27 heterozygous female parents was 19%, exactly as expected. All but one of the 27 individual cs progeny proportions fit the 19% expectation (Chi^2 test probability ≥ 0.05). That one progeny proportion was 3%, significantly different from the 19% expected (Chi^2 test probability = 0.02). However, in a set of 27 test crosses, up to one observation this extreme will occur by chance about 8% of the time. So results of crosses to detect heterozygous individuals fit well with expectations from a single-locus recessive model.

When 27 normal female parents genotyped as cs1 carriers (heterozygotes) were pairwise mated, and also crossed with individuals genotyped as cs1 non-carriers as a control, segregation of cs phenotypes in the progeny also fit well with a single-locus recessive model. In this case, mating two heterozygotes was expected to result in exactly 25% cs progeny. When an average of about 13 (10-15) plants of each of 29 progenies from matings of 18 unique parents identified as heterozygotes were planted in the field, cs progeny occurred as 114 of 367 plants, or 31%. This is slightly more than the expected frequency of 25%. However, all but one of the individual inter-heterozygote crosses' progeny fit the expectation of 25% cs ratio (P ≥ 0.05). In that single case, nine of 15 plants were mutants. As suggested for the crosses to identify heterozygote parents, this tendency toward an excess of cs phenotypes may reflect some form of fitness advantage of the mutant. As expected, control crosses for which either the maternal or paternal parent was a non-carrier never produced any mutant progeny.

Although the above results are consistent with a model of a single-locus recessive, bear in mind that these crosses were all made within the original crazy sepal population, PI 473166. This population may be fixed for epistatic genes necessary for expression of crazy sepal, so a more complex inheritance might be revealed when, for example, one would attempt introgression into cultivated tuberosum background. Thus, hybridization of cs1 heterozygotes with tuberosum haploids is a goal for future work. Future work might also include confirmation of the apparent superior seedset of heterozygous females in a bulk intermate of normals.

While the mutant phenotype vs wild type is qualitative (complete lack of stamens, pistils, and petals), the form of sepals varies among mutant individuals (ranging from small and light colored to larger, dark, and leaf-like)—an aspect of the mutant that also bears future investigation. This mutant may be a useful tool for the study of floral genetics in Solanum, might be an efficient marker to study rare genetic events, and would provide an absolute sterile that could have applications in breeding, especially if it could be artificially reversed. This mutant and other genetic stocks are freely available from USPG by contacting the author.

ACKNOWLEDGMENTS

LITERATURE CITED

Bisognin DA, DS Douches, L Buszka, G Bryan and D Wang. 2005. Mapping late blight resistance in Solanum microdontum Bitter. Crop Sci 45:340-345.

Ditta G, A Pinyopich, P Robles, S Pelaz and MF Yanofsky. 2004. The SEP4 gene of Arabidopsis thaliana functions in floral organ and meristem identity. Current Biol 14:1935-1940.

Eckardt NA. 2003. MADS monsters: controlling floral identity. The Plant Cell 15:803-806.

Hart JK and DJ Hannapel. 2002. In situ hybridization of the MADS-box gene POTM1 during potato floral development. J Exp Bot 53:465-471.

Krizek BA and JC Fletcher. 2005. Molecular mechanisms of flower development: an armchair guide. Nat Rev/Genet 6:688-698.